These primers functioned therefore as broad-range primers for bacteria. Sensitivity of the primers varied, and the primer pair with the highest sensitivity was used for studying the clinical specimens (otitis media samples). However, it was found out that this primer pair was not sufficiently sensitive, because it was not able to amplify bacterial DNA from clinical specimens that contained large amounts of human DNA.

For this reason new primer pairs that varied in terms of degeneration were synthesized (ordered from Sigma-Genosys, England, www.sigma-genosys.co.uk). The specificity and the sensitivity were studied by the previously described method, both with pure bacterial DNA and with DNA isolated from clinical specimens (Table 5). A functioning primer pair was the mixture of primers, which contains the sequences

CGTCCWGGKATGTAYATHGG (SEQ. ID. NR: 77) and
CCHACRCCRTGWAAWCCDCC (SEQ. ID. NR: 78),

which were named as gB1F (forward primer mixture) and gB2R (reverse primer mixture), respectively (Table 1), wherein

W represents base A or T,
K represents base G or T,
Y represents base C or T,
H represents base A or C or T,
R represents base A or G, and
D represents base A or G or T.

The conserved sequences of the first part of the *gyrB* and/or *parE* genes of all studied bacterial species were identified with this mixture of primers. It amplifies DNA from clinical samples, and has preserved sufficiently broad specificity, thus enabling the amplification of the *gyrB*/*parE* genes from all bacterial species causing respiratory tract infections (see Examples 6 and 7). In particular, this mixture of primers can be used to amplify the *gyrB*/*parE* genes of bacteria (Table 3) that are phylogenetically far from each other even in a situation where the sample includes large amounts of human DNA.

Claims

1. A diagnostic method for detecting and identifying bacterial species causing infections from a clinical sample, characterized by

a) amplifying DNA isolated from said clinical sample using a mixture of DNA primers that comprises sequences which hybridize with the sequences that originate from conserved regions of genes encoding topoisomerases, especially *gyrB/parE*, of bacterial species causing said infections, said sequences comprising sequences identified with SEQ. ID. NR: 76 and 77 or with reverse and/or complementary sequences thereof or functional fragments thereof,

b) contacting the amplified DNA with a desired combination of oligonucleotide probe sequences that hybridize under normal hybridization conditions with hyper-variable regions situated near said conserved regions of genes encoding topoisomerases, especially *gyrB/parE,* of bacterial species causing said infections, said sequences being bacterial species-specific under said hybridization conditions, and

c) detecting the formation of a possible hybridization complex.

2. The diagnostic method according to claim 1, characterized in that said infections causing bacterial species are bacterial species that cause respiratory tract infections.

3. The diagnostic method according to claim 1 or 2, characterized in that said hyper-variable region is the hyper-variable region of the gene encoding the *gyrB* and/or *parE* protein of a bacterial species selected from *Streptococcus pneumoniae, Streptococcus pyogenes, Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Escherichia coli, Moraxella catarrhalis, Legionella pneumophila*, and *Fusobacterium necrophorum.*

4. The diagnostic method according to any one of claims 1 to 3, characterized in that the length of oligonucleotide probe sequences used in step b) is 15 – 30, more preferably 20 – 30, and most preferably 21 – 25 nucleic acids.

5. The diagnostic method according to any one of claims 1 to 4, characterized in that said combination of oligonucleotide probe sequences comprises all or a portion of the sequences identified with SEQ. ID. NR: 1 to 69, and/or reverse or complementary sequences thereof, or functional fragments thereof.

6. The diagnostic method according to claim 5, characterized in that said combination of oligonucleotide probe sequences comprises all the sequences identified with SEQ ID. NR: 1 to 69.

7. The diagnostic method according to any one of claims 1 to 6, characterized in that said combination of oligonucleotide probe sequences is attached onto a solid support.

8. The diagnostic method according to claim 1, characterized in that the DNA isolated from the clinical sample in step a) is amplified using the polymerase chain reaction (PCR) and that the DNA amplified in step b) is contacted with bacterial species-specific oligonucleotide probes attached onto a solid support.

9. The diagnostic method according to claim 7 or 8, characterized in that said solid support is treated glass.

10. The diagnostic method according to claim 1, characterized in that suitably labeled nucleotides are used in the amplification of DNA isolated from a clinical sample in step a) to generate a detectable target strand.

11. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support, on which all bacterial species-specific oligonucleotide probes identified with SEQ. ID. NR: 1 to 69 and/or reverse or complementary sequences thereof have been attached.

12. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support on which specific oligonucleotide probe sequences detecting one specified bacterial species or a few specified bacterial species causing infections have been attached, said sequences being selected from sequences shown in Tables 4A and 4B and/or reversed or complementary sequences thereof.

13. The diagnostic method according to any one of claims 1 – 12, characterized in that the microarray technology is used in step c).

14. A DNA primer mixture, characterized by comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76

and 77 and/or reversed or complementary sequences thereof or functional fragments thereof.

15. An oligonucleotide sequence useful in the diagnosis of infection causing bacterial species, characterized in that it hybridizes under normal hybridization conditions with a sequence of a hyper-variable region that is bacterial species-specific and is situated near the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, said oligonucleotide sequence being one of the sequences identified with SEQ ID. NR: 1 to 69 and/or reverse or complementary sequences thereof functional fragments thereof.

16. The combination of oligonucleotide probe sequences useful in the diagnosis of infection causing bacterial species, characterized by comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or reverse or complementary sequences thereof or functional fragments thereof.

17. The combination of oligonucleotide probes according to claim 16, characterized by comprising all of the sequences identified with SEQ. ID. NR: 1 to 69.

18. The use of the combination of oligonucleotide probes according to claim 16 or 17 for the detection, identification, or classification of infection causing bacterial species.

19. The use of hyper-variable sequences situated near the conserved regions of topoisomerase genes, especially those encoding the *gyrB* and/or *parE* proteins, of infection causing bacterial species as species-specific hybridization probes.

20. The use of claim 19, characterized in that said infection causing bacterial species are respiratory tract infections causing bacterial species.

21. A diagnostic kit for use in the diagnosis of infection-causing bacteria, especially those causing respiratory tract infections, characterized by comprising

a) a DNA primer mixture comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the gyrB and/or parE proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76 and 77 and/or

reversed or complementary sequences thereof or functional fragments thereof.of the invention as defined above,

b) a combination of bacterial species-specific oligonucleotide probe sequences, optionally attached on a solid support, comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or reverse or complementary sequences thereof or functional fragments thereof.

c) positive and optionally negative control probe sequences, and optionally

d) reagents required in the amplification, hybridisation, purification washing, and/or detection steps.